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Increased Resistance to Tuberculosis in Mice Sensitized with Non-Specific Antigen

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Although there have been lots of investigations with reference to the mechanism of tuberculous immunity since the finding of the socalled Koch's phenomenon in 1891, the true mechanism has not yet been clarified. Some degree of acquired resistance is noted in the host which has been previously infected with living bacilli or injected with killed bacilli. It has not been clearly determined that this acquired resistance is directly connected with an actual immunological process in view of the antigen-antibody reaction. Although antibodies are proved in the serum of immune animals or humans in the form of precipitation or agglutination reactions, it has been almost definite that these anibodies have no direct connection with the defense mechanism of the host. Therefore, many authors have thought that immunity to tuberculosis occurs in the form of cellular defensive power. At this point, immunity is mixed up with allergy in tuberculosis. As a matter of fact, however, our serial investigations concerning the role of humoral factors in native and acquired resistance to tuberculosis have shown that there is certainly a humoral defensive power stimulated by immunizing procedures and that by further analysis this stimulated power to inhibit the growth of tubercle bacilli *in vivo* and *in vitro* is closely connected with the alteration of the A : G ratio in serum.

Recently Takeoka,[1] one of our associates, has demonstrated that the serum of animals treated by injection of bovine serum or typhoid vaccine ——immunologically non-specific antigens—— has a more powerful inhibiting effect on the growth of virulent tubercle bacilli *in vitro* than the control serum of non-treated animals, when tested by the ring method. This investigation suggests that the defensive power of body fluids of animals against tubercle bacilli may be stimulated by a non-specific mechanism as well as by specific immunity. It may be of great interest to examine whether or not animals treated by these non-specific procedures can be more resistant to tuberculous infection than non-treated control animals.

Recently, Dr. León[2] in Mexico produced a new synthetic tuberculous antigen and kindly sent it to our laboratory, requesting our examination of its immuno-

genicity. This antigen is composed of aluminum phosphate adsorbing polysaccaride extracted from BCG bacilli in combination with γ-globulin obtained from either rabbits's serum immunized with BCG or the serum from tuberculous patients. According to León, mice injectcd with this antigen into the tail vein 3 or 4 times at two week intervals could elongate their average survival time against tuberculous infection to 200 days, while non-treated control mice died in 124 days. He concluded that this demonstrated the apparent immunogenicity of this antigen.

The protecting activity of this specific antigen against tuberculous infection was compared with that of the non-specific treatments described above.

MATERIALS AND METHODS

SM mice (about 1.5 months after birth) are divided into four groups, as follows.

I : 19 mice immunized with León's antigen

II : 18 mice sensitized with typhoid vaccine

III : 19 mice sensitized with bovine serum

IV : 12 mice, non-treated controls

5 mice each from groups II and III were used to determine the antibody titer in the serum.

3 of group III died during sensitization because of toxicity of bovine serum or anaphylaxis.

The outline of the treatment in each group before the challenge with tubercle bacilli is shown in Fig. 1.

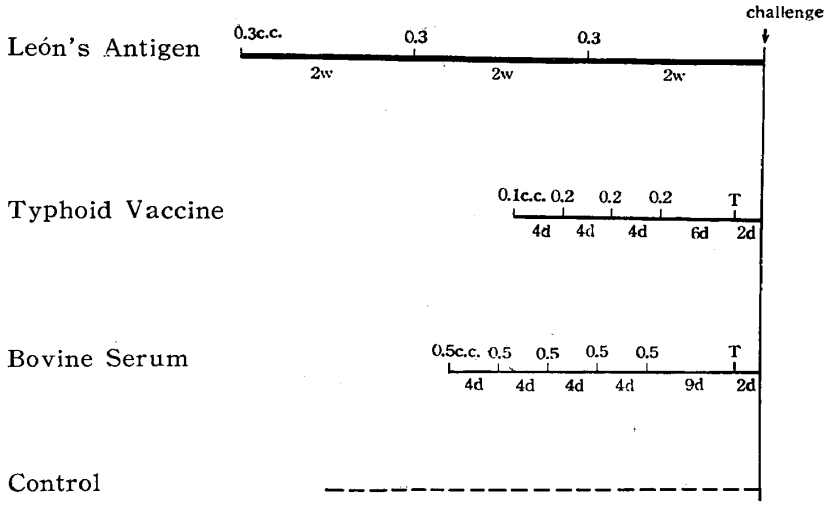

Fig. 1. The outline of the treatment in each group before the challenge with tubercle bacilli

T : The day on which the antibody titer was determined

In group I, 0.3 ml of Leóns antigen was injected into the tail vein 2 times at two week intervals, and 2 weeks after the last injection 0.1ml of a suspension of tubercle bacilli was injected into the tail vein.

In group II, 0.2 ml of typhoid vaccine was injected into the tail vein on the lst, 5th, 9th and 13th days, and the challenging bacilli were injected intravenously on the 2lst day.

In group III, 0.5 ml of bovine serum was injected intraperitoneally 5 times at 4 days intervals, and 11 days after the last injection tubercle bacilli were inoculated into the tail vein.

Group IV was an untreated control group.

The time of the start of these pretreatments were controled so that the challenge of tubercle bacilli could be done in the same day in all groups.

Tubercle bacilli of the H37Rv strain were used. The bacillary membrane cultured on glycerine-bouillon for 2 weeks was made into a suspension of 1 mg/ml concentration with physiological saline solution, and 0.1 ml of this suspension was injected into the tail vein.

Determination of the antibody titer was carried out using the serum collected and pooled from 5 mice by cutting the neck artery on the 6th day after the last sensitization in group II and on the 9th day in group III.

The Widal reaction was performed in group II, and the precipitation reaction using 100 fold diluted bovine serum in group III.

According to the description by León, León's vaccine was prepared as follows.

"A polysaccharide is extracted from *M. tuberculosis var. bovis* strain BCG, by suspending 1 mg. of semidried living cells per 100 ml of steril distilled water; the suspension is kept at 4~6°C during two weeks, centrifuged several times to get rid of the bacteria and three volumes of ethanol plus 3% sodium acetate are added to the supernatant. A precipitate is obtained that is redissolved in distilled water and precipitated in the same way three times when a pure polysaccharide is obtained. This polysaccharide has the properties of a specific incomplete antigen or hapten. It fixes the complement in the presence of rabbit anti-BCG serum or sera from human cases of tuberculosis; but it is unable to stimulate anti-body formation when injected parenterally to laboratory animals. This polysaccharide is immunologically combined in optimal proportions with immune gamma globulin from anti-BCG rabbit serum, and the complex polysaccharide-gamma-globulin so obtained is addsorbed to particles of aluminium phosphate by mixing, shaking and incubating at 37°C for 3 hours, equal parts of a dilution in steril distilled water of the complex polysaccharide-gamma-globulin, at a concentration of 1 : 20,000 of the polysaccharide, and 2 : 1000 of aluminium phosphate. The complex so obtained now has the properties of a specific complete antigen."

RESULTS

(1) Antibody Titer

In group II, a positive reaction was noted at 1 : 800 against typhoid, at 1 : 800 against para-typhoid A, and at 1 : 400 against para-typhoid B. No other increase in antibody titer was noted. In addition, it was noted that in the non-treated control mice, the Widal reaction was completely negative even at 1 : 25 concentration. Therefore, it may be reasonable to think that in mice the Widal titer can not go very high because of the innate characteristics of this animal species, and that a titer as high as 800 may have sufficient significance in the sensitization with typhoid vaccine.

In group III, there was a 2^6 precipitin titer. Antibody-titers in both groups were thought to indicate that sensitizations had given to the animals a fairly strong influence.

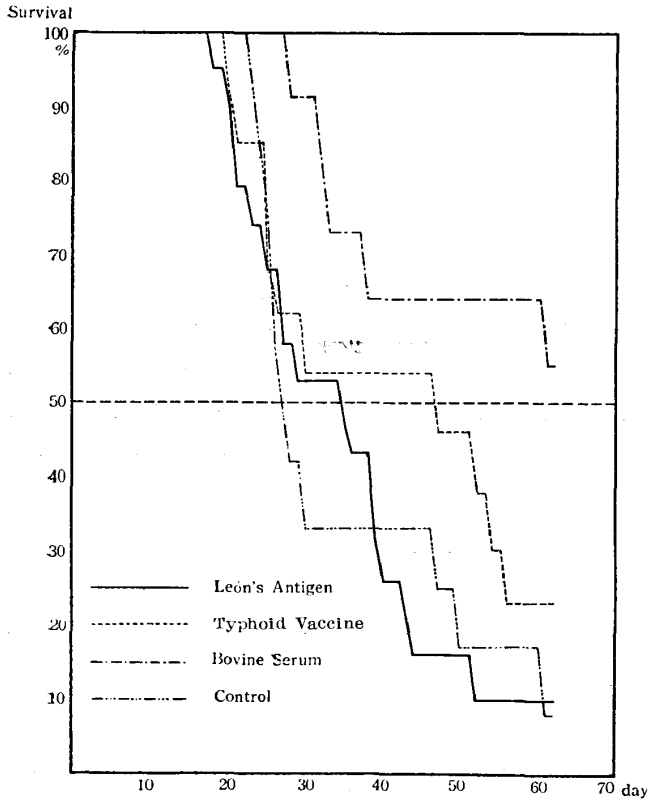

Fig. 2. Survival rate and time in mice treated with various antigens

(2) Survival Times

Fig. 2 shows the survival rate in percentages in the ordinate and the survival time in days in the abscissa. It is evident that the day when 50% of the deaths occurred, took the following order : group IV (control) shortest (27 days), group I (León antigen) 34.5 days, group II (Typhoid vaccine) 46.5 days and group III (Bovine serum) over 60 days. From the pattern of survival rate, no distinct difference between group I and group IV can be pointed out, but a relative slight but well-defined prolongation of life is seen in group II, and the most evident prolongation is in group III.

DISCUSSION

In order to determine the degree of immunogenic property using the survival time of mice as indicator, attention must be paid to the technique and conditions of the experiment. It would be desirable to make the experimetal

results accurate and significant to have a majority of the mice in a group die without large differences in survival time. For this purpose the selection of the strain of animals and bacilli, the mode of inoculation of bacilli, and the condition of the breeding of animals are important. In examining the effect of a chemotherapeutic agent it has been generally thought to be convenient for the control animals to die within a relatively short period after being challenged by bacilli, and the experimental results become inaccurate if average survival period is very long. In León's experiments the average survival time of the control group was 124 days and that of the immunized group 200 days. These experimental conditions may not be adequate, for the reason described above, for determining the immunogenicity. We considered this point and planned to keep the average survival time of control animals to under 50 days. As the results a slight prolonging effect of León's antigen was recognized in the length of time it took for 50% to die. However, there was some lack of uniformity of results also in this experiment showing that more animals in the control group remained alive in the late period of the experiment than in the immunized group, and from the stand point of average number of days of survival there was almost no difference between the two groups.

The following points may be considered as the cause of the lack of uniformity in this experiment. The H37Rv strain used in this experiment was less virulent than the Kurono strain which is usually used for testing chemotherapeutic agents, and, in addition, SM mice were less susceptible than DD mice, so the average survival period was somewhat prolonged.

Anyway, we could not confirm the ability of León's antigen to act as a powerful antituberculous vaccine, contrary to León's opinion. However, it may be that the vaccine changed in some of its properties during transportation from Mexico to Japan.

It is of great interest that non-specific sensitization using bovine serum or typhoid vaccine has given animals powerful and significant resistance to tuberculosis.

Non-specific resistance brought about by infection or vaccination with one species of bacteria against other kinds of pathogens has become one of the interesting problems in experimental biology. Nukada[3] and his associates reported on many of their experiments in which the injection of bacillary autolysates of one strain of bacteria (heterasate) is capable of protecting animals against infection by other strains of pathogens, including tubercle bacilli. Dubos and Schaedler[4] found that pretreatment with endotoxins of various origins including Salmonella typhosa influenced infections by various other strains of bacteria including tubercle bacilli and acted as a protection if the infection was not induced too early after the pretreatment. They[5][6] also observed that the

cellular components of mycobacteria acted either to protect against or to enhance infections of other sorts (staphylococuss aureus) according to the mode of treatment. Elberg, Schneider and Fong[7] found that there was a sort of cross-immunity between Brucella melitensis and Mycobacterium tuberculosis. Fenner[8] reported a similar observation between different strains of mycobacteria. In short, it may become more important to search for the non-specific mechamism of the protective power of animals in natural resistance or immunity.

In our laboratory,[9] investigation of the role of the protein fractions of the serum revealed that non-specific change ——reduction in the growth-promoting power of the albumin fraction and a change in the A : G ratio (albumin decreases and globulin increases)—— might be more significant than a specific change in the globulin fraction in the immunity to tuberculosis. Recently, Takeoka demonstrated that sensitization of rabbits with bovine serum or typhoid vaccine brought about in the protein fraction of the serum the same change of effect on growth of tubercle bacilli *in vitro* as was found in the serum of immune animals.

In this investigation, it has been clearly demonstrated that mice were also influenced by these non-specific sensitizations so as to increase their resistance to tuberculosis *in vivo*.

It appears from these results that the non-specific mechanism plays a more important role than specific immunological change in tuberculous immunity, at least in humoral immunity.

Further experiments using other specific vaccines (BCG or killed vaccine) may be necessary in order to reach a conclusion.

SUMMARY

Mice (SM strain) were sensitized non-specifically with either bovine serum or typhoid vaccine and the immunogenicity of these treatments to tuberculosis was compared with that of a specfic antituberculous vaccine prepared by Dr. León in Mexico, using the survival time of amimals as an indicator.

(1) Contrary to León's opinion, the presence of a significant immunogenic property could not be confirmed in León's vaccine.

(2) An apparent increase in resistance is observed in animals treated with bovine serum and to lesser extent with typhoid vaccine.

(3) A non-specific mechanism can be thought to play a very important role in addition to the specific immunological mechanisms in antituberculous immunity.

Acknowledgment : We express thanks to Dr. León for his kindness of sending us his vaccine.

REFERENCES

1) Takeoka, A. : Japan. J. Tuberc., **6**, 127, 1958.
2) León, A. P. : Personal communication, 1958.
3) Nukada, S., Kimura, T., Yasaki, Y. and Utsunomiya, S. : The Tokyo Journal of Medical Sciences, **61**, 183, 1953.
4) Dubos, R. J. and Schaedler, R. W. : J. Exper. Med., **104**, 53, 1956.
5) Dubos, R. J. and Schaedler, R. W. : J. Exper. Med., **106**, 703, 1957.
6) Schaedler, R. W. and Dubos, R. J. : J. Exper. Med., **106**, 719, 1957.
7) Elberg, S. S., Schneider, P. and Fong, J. : J. Exper. Med., **106**, 545, 1957.
8) Fenner, F. : Am. Rev. Tuberc., **76**, 76, 1957.
9) Oshima, S., Fujita, Y., Takeoka, A., Nakajima, M. and Tsuji, S. : Am. Rev. Tuberc., **78**, 884, 1958.